Identification of a Nonsteroidal Liver X Receptor Agonist through Parallel Array Synthesis of Tertiary Amines

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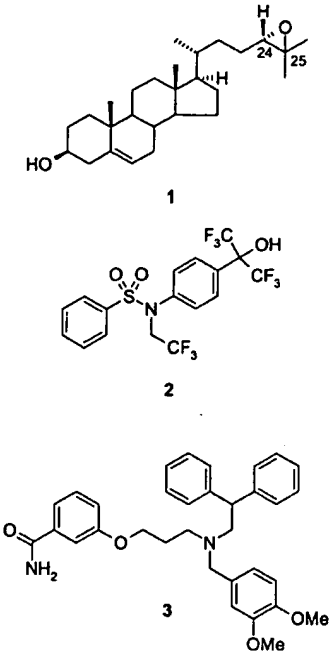
Abstract: A potent, selective, orally active LXR agonist was identified from focused libraries of tertiary amines. GW3965 (**12**) recruits the steroid receptor coactivator 1 to human LXRα in a cell-free ligand-sensing assay with an EC_{50} of 125 nM and profiles as a full agonist on hLXRα and hLXRβ in cell-based reporter gene assays with EC_{50}'s of 190 and 30 nM, respectively. After oral dosing at 10 mg/kg to C57BL/6 mice, **12** increased expression of the reverse cholesterol transporter ABCA1 in the small intestine and peripheral macrophages and increased the plasma concentrations of HDL cholesterol by 30%. **12** will be a valuable chemical tool to investigate the role of LXR in the regulation of reverse cholesterol transport and lipid metabolism.

The increased incidence of cardiovascular disease (CVD) in westernized nations has been linked to increased dietary intake of cholesterol and saturated fats and an increase in low-density lipoprotein (LDL) particles.[1] Accumulation of small, dense LDL particles in the arterial wall leads to the formation of cholesterol-laden foam cells, which are the hallmark of coronary atherosclerosis, and activation of the immune system.[2] Although cholesterol-lowering drugs, such as statins, reduce the incidence of CVD in patients with high circulating levels of LDL cholesterol (LDLc), atherosclerosis also afflicts individuals with relatively normal levels of LDLc. In contrast to LDL, the levels of high-density lipoprotein (HDL) particles are inversely related to the incidence of CVD.[3] The protective role of HDL may result from its role in mediating "reverse cholesterol transport" whereby cholesterol is transported from peripheral cells, including the macrophage-derived foam cells, back to the liver.[4] Thus, agents that promote reverse cholesterol transport by raising circulating levels of HDL could provide an alternative therapeutic option for the prevention of atherosclerotic CVD.[5]

A pivotal step in the process of reverse cholesterol transport is the efflux of free cholesterol from peripheral tissues to nascent HDL particles. Mutations in the cholesterol transporter adenosine triphosphate binding cassette (ABC) A1 were recently identified as the genetic basis of Tangier disease (TD), a condition characterized by an absence of plasma HDLc.[6–8] TD patients show a decreased capacity to effect cholesterol efflux from peripheral tissues and also develop premature atherosclerosis.[9] Notably, targeted disruption of the ABC1 gene in mice results in HDL deficiency and increased foam cell formation compared to wild-type mice.[10] Thus, drugs that upregulate ABCA1 expression could provide a method for promoting reverse cholesterol transport and preventing CVD.[4]

Chart 1. Structures of Epoxycholesterol (**1**), T0901317 (**2**), and Partial Agonist **3**



The liver X receptors, LXRα (NR1H3) and LXRβ (NR1H2), are oxysterol-activated transcription factors that belong to the nuclear hormone receptor superfamily.[11,12] 24(*S*),25-Epoxycholesterol (EPC, **1**) may be an endogenous ligand for LXRα in the liver (Chart 1).[13,14] Upon cholesterol feeding, the hepatic levels of **1** are raised in rats to levels consistent with its putative role as a natural LXRα agonist.[15–17] The LXRs function as heterodimers with the 9-cis retinoic acid receptor RXR (NR2B) to regulate the expression of the ABCA1 gene.[18–21] A nonsteroidal LXR agonist, T0901317 (**2**), was recently reported to increase ABCA1 expression and raise HDLc levels in mice.[20,22,23] In this report we describe the identification of a novel chemical series of LXR agonists through solid-phase parallel array synthesis of tertiary amines. These compounds may provide leads for the development of drugs to increase reverse cholesterol transport.

Tertiary amine **3** was identified from a high-throughput screen of the GlaxoSmithKline compound file using a cell-free ligand-sensing assay (LiSA) for human LXRα. The LXRα LiSA measures the ligand-dependent recruitment of a 24 amino acid fragment of the steroid receptor coactivator 1 (SRC1) to the ligand-binding domain of the receptor.[24] Tertiary amine **3** showed an EC_{50} of 260 nM

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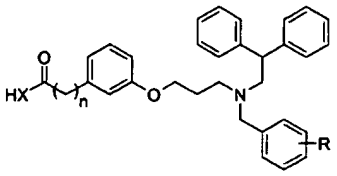
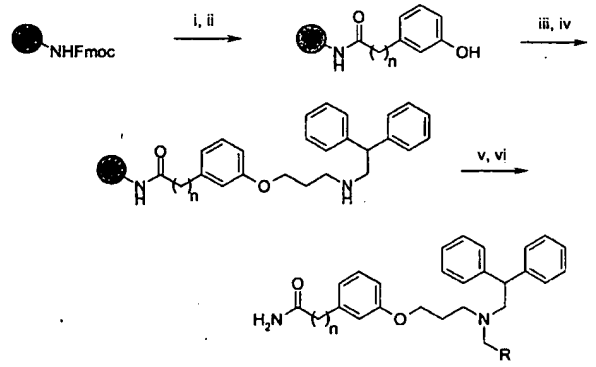
Table 1. LXR Activity of Tertiary Amines[a]



compd	*n*	X	R	LXRα/SRC1 LiSA EC50 (nM)	LXRα/SRC1 LiSA RE	LXRα-GAL4 EC50 (nM)	LXRα-GAL4 RE
1			24(*S*),25-epoxy-cholesterol	95 ± 4	1.0	3000 ± 750	1.0
2			T0901317	60 ± 4	1.0	85 ± 10	2.5
3	0	NH	3,4-dimethoxy	260 ± 60	0.2	nt	nt
4	1	NH	4-methoxy	260 ± 10	0.8	ia	
5	1	O	4-methoxy	860 ± 50	0.8	8000 ± 3200	0.9
6	1	NH	2,4-dimethoxy	660 ± 10	0.7	4000 ± 1300	0.6
7	1	NH	3-fluoro-4-methoxy	250 ± 40	0.7	910 ± 20	0.5
8	1	NH	2-fluoro-4-methoxy	190 ± 50	0.9	700 ± 220	0.4
9	1	NH	3-trifluoromethyl	85 ± 20	0.7	650 ± 300	0.3
10	1	NH	4-fluoro-3-trifluoro-methyl	85 ± 5	0.9	945 ± 365	0.4
11	1	NH	2-chloro-3-trifluoro-methyl	45 ± 10	1.1	425 ± 115	1.5
12	1	O	2-chloro-3-trifluoro-methyl	125 ± 20	1.0	190 ± 30	1.7

[a] EC_{50} = concentration of compound that leads to half-maximal activity ± standard error, $n = 3$. RE = relative efficacy compared to 24(*S*),25-epoxycholesterol (**1**). nt = not tested. ia = inactive at 10 μM.

Scheme 1[a]



[a] Reagents: (i) 20% piperidine, DMF; (ii) four phenolic acids, HATU, 2,6-lutidine, NMP; (iii) 3-bromopropanol, DIAD, Ph_3P, toluene, 0 → 25 °C; (iv) 2.0 M diphenethylamine, DMSO; (v) RCHO, $NaHB(OAc)_3$, 8% AcOH/DMF; (vi) 10% TFA, DCM.

on LXRα but was only partially effective at recruiting the SRC1 protein compared to EPC (**1**) (RE = 17%, Table 1). To improve the efficacy of **3**, a solid-phase synthesis of tertiary amines was developed that would allow exploration of the benzamide and benzylamine functionalities (Scheme 1). Among the solid-phase linkers that were evaluated were the Sasrin linker, which yields a carboxylic acid upon cleavage of the resin, and the Rink linker, which yields the corresponding amide. The Rink amide linker was chosen for initial array synthesis, since it provided the highest overall yield and purity of final products in preliminary studies (data not shown). Four commercially available phenolic acids were loaded onto solid support and subsequently reacted with 3-bromopropanol under Mitsunobu conditions. The resin-bound bromides were treated with a solution of diphenylethylamine in DMSO, and the resulting secondary amines were subjected to reductive amination with 30 commercially available benzaldehydes. Cleavage from the solid support provided an array of 120 tertiary amines in approximately 83% average purity and 50% overall yield as determined by HPLC and chemiluminescent nitrogen detection (CLND). The array of tertiary amines was screened at a concentration of 1 μM using the LXRα/SRC1 LiSA. Phenylacetamide **4** was identified as a potent LXRα ligand with increased efficacy for recruitment of the SRC1 peptide compared to **3** (Table 1). Unfortunately, **4** was inactive at doses up to 10 μM in cell-based LXRα reporter gene assays[13] (Table 1). In an attempt to change the physical properties of amide **4**, the corresponding carboxylic acid **5** was synthesized using Sasrin resin in place of Rink amide resin. Although **5** was less potent in the LXRα/SRC1 LiSA, it showed improved activity with an EC_{50} of ~8 μM in the cell-based reporter gene assay (Table 1).

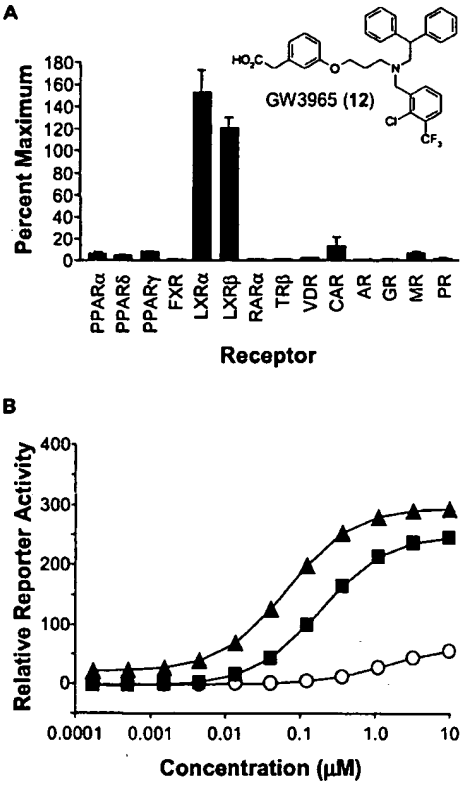


Figure 1. (A) Activity of **12** (1 μM) on hPPARα-GAL4, hPPARδ-GAL4, hPPARγ-GAL4, hFXR-GAL4, hLXRα-GAL4, hLXRβ-GAL4, hRARα-GAL4, hTRβ-GAL4, hVDR-GAL4, full-length hCAR, full-length hAR, full-length hGR, full-length hMR, and full-length hPR. Percent maximum is the activity relative to a positive control for each receptor. Data are representative of two or more independent experiments. (B) Dose–response analysis of **12** on hLXRα-GAL4 (■), hLXRβ-GAL4 (▲), and hPXR-GAL4 (○). Reporter activity is relative to EPC (**1**) for LXR and rifampicin for PXR. Data are representative of four independent experiments.

In an effort to further increase the LXRα activity of the tertiary amines, an array of 1280 carboxamides were synthesized using Rink amide linker and screened for activity in the LXRα/SRC1 LiSA at 1 μM. Six carboxamides (**6–11**) were identified from the array with activity less than 1 μM in the LXRα/SRC1 LiSA (Table 1). Several of these analogues contained a *m*-trifluoromethyl functionality in the benzylamine substituent (**9–11**), with the 2-chloro-3-trifluoromethylbenzylamine **11** identified as the most potent analogue with an EC_{50}

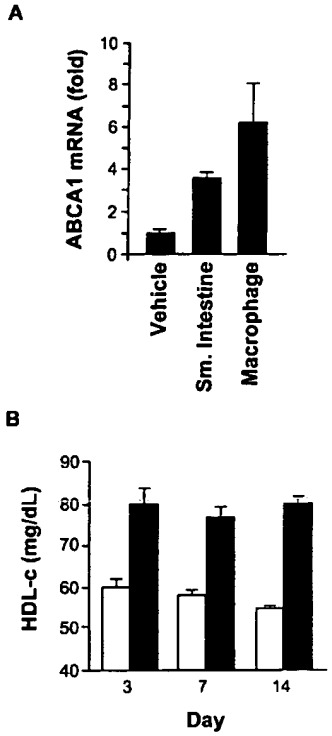


Figure 2. Effects of dosing **12** at 10 mg/kg bid to C57BL/6 mice ($n = 3$) on ABC1 expression levels in small intestine and peripheral macrophages (A) and plasma levels of HDLc (B).

of 45 nM in the LXRα/SRC1 LiSA. As was seen in the earlier series of carboxamides, **11** showed a reduction in potency when tested in the cell-based LXRα-GAL4 reporter gene assay. Since cellular potency had been improved through conversion of amide **4** to carboxylic acid **5**, the corresponding carboxylic acid **12** was synthesized using the Sasrin linker. Carboxylic acid **12** showed an EC_{50} of 125 nM in the LXRα/SRC1 LiSA with comparable efficacy to EPC (**1**) for recruitment of the SRC1 peptide. To our delight, **12** maintained its potency in the LXRα cell-based reporter gene assay with an EC_{50} of 190 nM (Table 1, Figure 1B). When screened against a panel of nuclear receptors, **12** showed cross reactivity with only LXRβ (Figure 1A) and the pregnane X receptor (PXR) (data not shown). Full dose–response analysis on LXRβ- and PXR-GAL4 chimeras showed **12** was >10-fold selective for activation of LXR compared to PXR (Figure 1B). Thus, carboxylic acid **12** is a potent LXR agonist with good cellular activity and excellent selectivity over other nuclear receptors.

In mice, **12** showed 70% oral bioavailability with C_{max} = 12.7 μg/mL and $t_{1/2}$ = 2 h after dosing at 10 mg/kg. Analysis of the pharmacokinetic data indicated that the serum levels of **12** were 5-fold above its EC_{50} in cells for up to 7 h after dosing. The pharmacological activity of **12** was evaluated in C57BL/6 mice by dosing at 10 mg/kg bid for 14 days. By day 3, ABCA1 expression was increased 8-fold in the small intestine and 7-fold in peripheral macrophages (Figure 2A), while plasma levels of HDLc increased 30% at day 3 and was maintained until day 14 (Figure 2B). Thus, **12** is an orally active LXR agonist that upregulates ABCA1 expression and raises circulating levels of HDL in C57BL/6 mice.

In summary, carboxylic acid **12** has been identified through parallel array synthesis of tertiary amines as a potent LXR agonist with selectivity over other nuclear receptors including PXR. Carboxylic acid **12** is active on LXRα and LXRβ in cell-based reporter gene assays and shows oral bioavailability in mice with plasma concentrations reaching 5-fold above the EC_{50} for LXR activation. Moreover, **12** raises plasma levels of HDLc in C57BL/6 mice and increases ABCA1 expression in multiple tissues that are known to be involved in the process of reverse cholesterol transport. GW3965 (**12**) is structurally distinct from the oxysterol[24] and sulfonamide[20,22,23] classes of LXR agonists and represents a promising lead for the development of drugs to prevent atherosclerotic CVD.

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Supporting Information Available: Experimental procedures for the research described in this letter. This material is available free of charge via the Internet at http://pubs.acs.org.

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Rapid Purification of Small Molecule Libraries by Ion Exchange Chromatography

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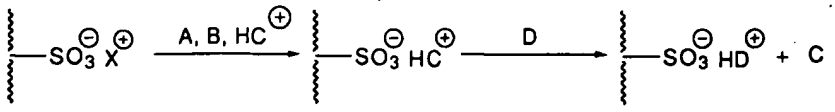
Abstract: Amines and acylated amines are synthesized in traditional solution phase reactions, then rapidly purified by ion exchange chromatography to yield pure products. In some instances, impurities devoid of ionizable functionality can be covalently modified prior to purification. The generic purification sequence is applicable to a variety of reactions, and is amenable to automation with commercially available equipment.

The generation of small molecule libraries for screening against biological targets has emerged as an area of intense interest in the pharmaceutical industry. These libraries have largely been synthesized using solid phase organic synthesis (SPOS) to minimize side products, expedite reaction work-up, and facilitate the application of automation.[1] More recently, workers have examined alternatives to SPOS for the production of large numbers of high purity compounds in parallel through a variety of techniques such as the use of solid supported reagents,[2,3] liquid/liquid extraction of solution phase reactions,[4] and fluorinated reagents for work-up simplification.[5] We have developed methodology for the expedited work-up and purification of traditional solution phase reactions using solid supported scavenging reagents.[6] Ideally, expedited synthesis methodology should be 1) applicable in a broad range of reactions; 2) tolerant of a variety of substrates within a reaction class; 3) amenable to automated synthesis. We now wish to present our results on the application of ion exchange chromatography in the expedited work-up and purification of organic molecules synthesized in solution, and in the automated construction of small molecule libraries.

Figure 1



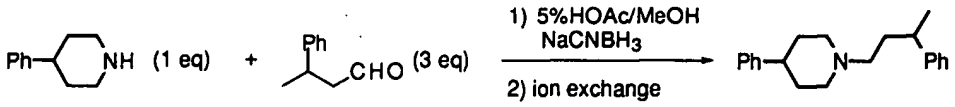
Strong Cation Exchange Column

The advantage of ion exchange chromatography over more traditional small molecule purification modes such as flash chromatography or HPLC is that one can reliably predict the elution characteristics of a broad range of molecules solely by the presence or absence of an ionizable site in the molecule. As illustrated simply in **Figure 1**, neutral molecules A and B pass through a sulfonic acid based cation exchange column, while protonated molecule C is retained. Reaction mixtures produced under a variety of conditions can be purified identically provided the desired products are either the only ionizable materials or the only non-ionizable materials

in solution, an ideal scenario for automated reaction purification. Ion exchange chromatography has been employed for many years in a wide variety of applications,[7] such as water purification,[8] protein purification,[9] and serum analysis.[10] Its potential as a method of purifying small organic molecules, however, has been largely unexplored, the principal application in this area being the purification of extremely hydrophilic molecules such as peptides and amino acids which can be difficult to purify by other means.[11,12]

Our initial experiment was the purification of a reductive amination reaction mixture using cation exchange chromatography (**Scheme 1**). In an illustrative example, 4-phenylpiperidine was combined with a 3-fold excess of 3-phenylbutyraldehyde in 5% acetic acid in methanol as solvent, and treated with an excess of sodium cyanoborohydride. When no starting secondary amine remained by TLC, the reaction mixture was poured over a Varian strong cation exchange (SCX) column.[13] The column was rinsed with methanol to remove neutral impurities, then treated with a 2M solution of anhydrous ammonia in methanol to elute the product tertiary amine in 83% yield and 86% purity by HPLC analysis. The method of purification allows for the addition of a large excess of one reagent (in this case aldehyde) to drive the reaction to completion, as in solid phase synthesis, without fear of work-up complications. Thus purity and yield of the final product are high.

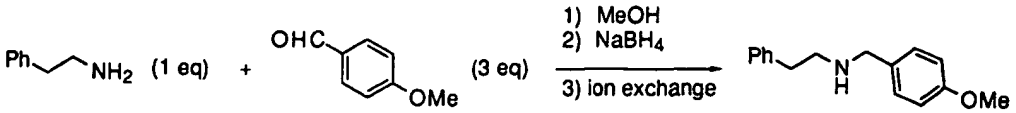
Scheme 1



yield: 83%
HPLC purity:86%

Similarly, secondary amines can be synthesized from the corresponding primary amines and aldehydes by employing a large excess of aldehyde in methanol to form imine nearly quantitatively with respect to primary amine, then reducing the mixture with sodium borohydride and passing the resulting solution over an SCX column (**Scheme 2**). These reductive amination procedures have been used in our labs to rapidly synthesize hundreds of secondary and tertiary amines in high purity.

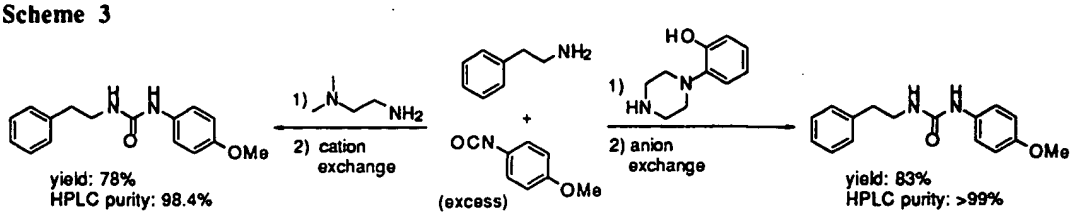
Scheme 2



yield: 86%
HPLC purity: 97%

We have also explored the possibility of covalently trapping a non-ionizable impurity with a solution phase scavenger to create an ionizable impurity for removal by ion exchange. The concept of solution phase scavengers is complimentary to our earlier work on solid phase covalent scavengers,[6] and is amenable to both anion and cation exchange chromatography depending on the scavenger employed. As an illustration, phenethylamine was reacted with 1.25 equivalents of 4-methoxyphenylisocyanate to form product urea plus starting isocyanate (**Scheme 3**). The isocyanate impurity was removed by quenching either with N,N-dimethylaminoethylamine followed by cation exchange, or by quenching with 1-(2-hydroxyphenyl)piperazine followed by anion exchange.[14,15]

Scheme 3



Since the ability to utilize ion exchange chromatography as a separation method relies on the nature of the products formed and is nearly independent of the reaction conditions, reactions conducted under a variety of conditions can be purified in a nearly identical manner. This is an ideal scenario for the application of simple robotics for purification. As an illustration, we synthesized three secondary amines by reductive amination using ion exchange purification (amine **1** from p-tolualdehyde and 3,3-diphenylpropylamine: 92% purity by HPLC; amine **2** from α-methylphenylacetaldehyde and 4-fluorobenzylamine: 94%; amine **3** from α-methylphenylacetaldehyde and 3,3-diphenylpropylamine: 91%) and subjected each with no further purification to reaction in a 3 x 3 matrix with three different substrates (aldehyde **A**, epoxide **B**, and isocyanate **C**). Each substrate required different reaction conditions: reductive amination with an aldehyde in acidic methanol, epoxide opening in methanol under neutral conditions, and acylation in dry chloroform (**Figure 2**). An excess of reagents was employed in such a way as to ensure that the desired product was the only ionizable component (for alkylation with substrates **A** or **B**) or the only non-ionizable component (for acylation with isocyanate **C**) present in the final reaction mixture. Thus, for reductive amination a two-fold excess of aldehyde **A** was employed, for epoxide opening a two-fold excess of epoxide **B** was employed, and for acylation with **C** a two fold excess of amines **1-3** was employed. When the reactions were complete by TLC as judged by disappearance of the limiting reagent, the nine solutions were subjected to ion exchange in an automated fashion using a commercially available Hamilton Microlab 2200 robot with SPE capability to separate ionizable from non-ionizable materials. The robot successfully purified all nine reactions independent of reaction conditions to a purity after two steps of greater than 80% in all cases and in excess of 90% in the majority of cases, with yields ranging from 71-92%.[16]

Figure 2: 3x3 matrix of reactions purified by ion exchange[a, b]

	1	2	3
A	yield: 72% HPLC purity: 98%	yield: 84% HPLC purity: 88%	yield: 71% HPLC purity: 95%
B	yield: 85% HPLC purity: 99%	yield: 81% HPLC purity: 80%	yield: 80% HPLC purity: 93%
C	yield: 90% HPLC purity: 91%	yield: 92% HPLC purity: 86%	yield: 87% HPLC purity: 87%

[a] purities listed are for the two step sequence with only ion exchange purification

[b] HPLC conditions: NovaPak 3.9 x 150 mm C18 column, 50-100% acetonitrile/0.1% TFA, 20 min.

In summary, we have explored the use of ion exchange chromatography for the automated purification of a variety of amine functionalizations and found it to be an effective method for the synthesis of high purity small molecule libraries in an automated fashion. Further applications of ion exchange chromatography for library synthesis will be reported in due course.

General Procedure for Reductive Amination: To a 4-mL vial was added 4-phenylpiperidine (20 mg, 0.124 mmol), 3-phenylbutyraldehyde (55 mg, 0.37 mmol), and 250 µL 10% acetic acid in methanol. To this solution was added 250 µL of a 1N solution of sodium cyanoborohydride in methanol. The vial was sealed and shaken at room temperature for 4 hours. The solution was applied to a 500 mg SCX column (Varian), and the column flushed with 3 mL methanol. The product was then eluted with 1 mL of 2 M ammonia in methanol to give 30 mg (83% yield) of the desired tertiary amine.

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11. Some workers have explored the use of ion exchange resins in the production of combinatorial libraries; see Gayo, L. M.; Suto, M. J. *Tetrahedron Lett.* **1997,** *38*, 513-516; Parlow, J. J. *Tetrahedron Lett.* **1996,** *37*, 5257-5260; Lawrence, R. M.; Biller, S. A.; Fryszman, O. M.; Poss, M. A.; Weller, H. A. A Simple Procedure for the Automated Solution Phase Synthesis and Purification of Non-Peptidic Amides. In *Conference on Molecular Diversity and Combinatorial Chemistry*; January 28, 1996: San Diego, CA, 1996.
12. For a recent and somewhat novel example of the use of solid phase extraction in the purification of combinatorial libraries, see: Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996,** *37*, 6961-6964.
13. Varian SCX columns are sulfonic acid residues covalently linked to silica gel. These prepacked columns are particularly convenient in that they readily fit off the shelf automated solid phase extraction equipment.
14. Analytical data for urea: ^{1}H NMR (300 MHz, $CDCl_3/CD_4OD$): 7.1-7.3 (m, 7H); 6.84 (d, 2H, J=9 Hz); 3.81 (s, 3H); 3.50 (t, 2H, J=6.8 Hz); 2.83 (t, 2H, J=6.8 Hz). MS (M+1): 271.1.
15. We have found it necessary to purify commercially available 1-(2-hydroxyphenyl)piperazine by flash chromatography in order to achieve optimal results
16. All compounds gave mass spectral data consistent with the desired structure.

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